DNA Enzyme Data Collection

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SUMMARY
Progress toward the structure determination of a mechanistically relevant conformation of a DNA enzyme is presented. The structure of a particular RNA:DNA construct of the 10-23 DNA enzyme was determined at 3.0 Å resolution [1]. This structure revealed that the complex had rearranged during crystallization, forming a four-way junction dependent upon a specifically bound metal ion. This result directed the development of a combinatorial crystallization screen designed to capture a functionally relevant conformation of the 10-23 DNA enzyme. This approach has led to ~40 new crystal forms, at least one of which diffracts to 2.8 Å resolution. The research effort is now poised to determine the structure of the active form of the 10-23 DNA enzyme.

In order to capture an active conformation of the DNA enzyme and prevent rearrangement during crystallization, additional experiments were carried out using constructs in which the 5′ and 3′ stems of the complex were either 8, 9, or 10 base pairs in length. It is expected that the stability of both stems is comparable to, or exceeds that of the 8 base-pair DNA:DNA duplex that forms in the 2:2 complex, thus preventing a significant fraction of the 1:1 complexes from rearranging to the 2:2 form. This is expected to favor the capture of the 1:1 complex within a crystal lattice.

COMBINATORIAL CRYSTALLIZATION SCREEN
For crystallization screening, a combinatorial strategy was used that takes advantage of the bimolecular nature of the RNA:DNA complex [2]. The length of the 5′ and 3′ arms of both the DNA enzyme and RNA substrate were systematically varied for lengths of 8, 9, or 10 residues, creating $3^4 = 81$ sequence variants. All 9 DNA and 9 RNA molecules were synthesized and used in crystallization trials. For each of the 81 possible DNA:RNA complexes, 48 crystallization conditions were set up, resulting in 3,888 unique combinations. The set of 48 crystallization conditions that was employed was based on previous experiments with the 10-23 DNA enzyme [1].

The concentration of each oligonucleotide in the complex was typically 0.4 mM. 20 nmoles of RNA:DNA complex was the minimal amount required to carry out a screen of 48 crystallization drops. Crystallization trials were carried out by vapor diffusion using the sitting drop method. The optimal volume of the drops was found to be 2 μl. Although the small drop volume increased the rate of nucleation, it allowed more experiments to be conducted with a given sample. 1 μl of nucleic acid solution was mixed with 1 μl of the reservoir solution and placed on a concave plastic pedestal in a 24-well plate. The volume of the reservoir was 1 ml. In order to prevent the evaporation of low molecular weight alcohols present in several of the crystallization solutions, each plate was tightly sealed with clear plastic tape. The plates (162) were maintained at 24.5 °C in a constant temperature incubator for at least two weeks. Molecules with favorable sequences crystallized within ~3 days. The droplets were evaluated using a binocular microscope (40x) equipped with polarizing filters to enable observation of crystal birefringence.

DIFFRACTION QUALITY CRYTALS
Altogether, 40 new crystal forms were observed [2]. Each of these crystal forms occurred with a unique morphology under a particular set of crystallization conditions and all were large enough for data collection. At least 20 of the 40 forms are birefringent, an indication of their crystalline

order. In addition to these 40 forms, 20 of the complexes yielded small or twinned crystals under one or more of the 48 conditions that were tested.

The diffraction properties of crystals were evaluated at room temperature using CuKα radiation from a Ru200 rotating anode x-ray generator equipped with a graphite monochromator and Siemens multiwire area detector. Crystals that showed the best diffraction with this x-ray source at room temperature (~5 Å resolution) were further evaluated at 100 K using synchrotron radiation. Depending on the composition of the crystallization mixture, the crystals were either frozen directly in a stream of cold N_2 gas, or were transferred briefly to a synthetic mother liquor containing increasing concentrations of cryoprotectant, frozen in liquid N_2 and then transferred to the x-ray beam. Typical cryoprotectants employed in the latter method were MPD and glycerol added in 10% increments to the original mother liquor.

Initial evaluation of 12 of the crystal forms, selected on the basis of their size, birefringence, and diffraction properties at room temperature, was carried out on beam line 5.0.2 at the Advanced Light Source (ALS) at Berkeley. There are at least two crystal forms that diffracted to ~3 Å resolution. Large rhombic plate crystals of the '99/89' complex showed diffraction to 2.8 Å resolution. The space group of these crystals is I222 or $I2_12_12_1$, with unit cell dimensions a = 61.3, b = 80.5 and c = 93.1 Å, as determined by indexing of both the ALS and room temperature data. For this unit cell the Matthews' coefficient is ~3.7 $Å^3$/dalton, assuming one RNA:DNA complex (~15 kD) in the asymmetric unit. Large hexagonal crystals of the '910/910' complex also showed diffraction to 2.8 Å resolution. Indexing of images from the ALS and room temperature data indicated that these crystals are orthorhombic. In addition, two crystal forms of the '910/1010' complex diffracted to 3.5–3.6 Å, and a crystal form of the '910/89' complex diffracted to 3.6 Å. In contrast, crystals of the '99/1010' complex, differing by only a single residue from the '910/1010' complex, diffracted to only ~10 Å. Because all of the crystal forms contain complexes with at least 8 base pairs in both stems, they are expected to exist as a 1:1 RNA:DNA complex.

REFERENCES

1. J. Nowakowski, P. Shim, G. S. Prasad, C. D. Stout and G. F. Joyce, Nat. Struct. Biol. **6**, 151-156 (1999).
2. J. Nowakowski, P. Shim, G. F. Joyce and C. D. Stout, J. Mol. Biol., submitted (1999).

This research supported by the Skaggs Institute for Chemical Biology.

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